# NONCLASSICAL KINETICS FOR ENZYMATIC REACTIONS IN TWO-DIMENSIONAL MEDIA

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*Abstract.* Monte-Carlo simulations of Michaelis-Menten enzymatic reactions occurring in two-dimensional unobstructed media reveal non-classical kinetics reflected by time dependent rate coefficients. A comparison between different equations proposed to describe rate coefficient time dependence is made and the initial conditions influence is also analyzed.

*Key words:* enzymatic reactions, rate coefficients, two-dimensional media, anomalous diffusion.

## INTRODUCTION

Among the most important chemical reactions for living organisms are enzymatic reactions. Experimental studies concerning their kinetics are usually conducted under well stirring conditions and for a small total amount of reactants in solution. Depending on experimental conditions, a connective stirring cannot always be achieved for reactants but there is still a diffusional stirring (also called "self-diffusion"). Also, the chemistry of biological organisms (*in vivo*) is fundamentally different from that in test tubes (*in vitro*) largely due to macromolecular crowding conditions [7]. Under crowding conditions and/or topological constraints, diffusional stirring may also be highly inefficient [2]. In such conditions we must take into account the interactions between all the molecules present in reaction medium and these interactions strongly affect the diffusion process and induce changes in the rates and equilibrium constants [7].

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Under crowding and/or spatially constraints classical kinetics has been found to be unsatisfactory and different non-classical approaches have been proposed to describe it: deterministic approach [5], power-law approach [9], fractal approach [2, 11] and stochastic approach [12]. Within this study we focus on fractal approach which assumes a temporal dependence for rate coefficient of second or higher order reactions [1, 2, 11, 13] and we compare different equations describing time dependence of rate coefficient in order to appreciate which of them is the most advantageous. Rate coefficients are obtained through Monte Carlo simulations of enzymatic reactions following a Michaelis-Menten mechanism in two-dimensional (2D) media. We also analyze the effect of initial concentrations of the substrate molecules (S), respectively of enzyme (E) molecules on the rate coefficients.

## METHOD

### DIFFUSION CONTROLLED REACTIONS IN TWO-DIMENSIONAL MEDIA

Theoretical and also experimental studies demonstrate that enzymatic reactions, and all diffusion controlled reactions, are strongly affected by spatial dimension in which they occur [1, 2, 4, 6–13]. Time dependence of the rate coefficient for reactions in 2D and crowded media has been explained in terms of fractal-like kinetics by Kopelman in 1986 [7]. He proposed the following equation to describe rate coefficient behavior

$$k(t) = k_0 t^{-h} \tag{1}$$

with $0 \leq h \leq 1$ and $t > 0$. In this equation $k_0$ coincides with the rate constant for classical kinetics (when $h = 0$, we obtain $k = k_0$) and $h$ is called the fractal parameter being dependent on topological dimension of the medium and on crowding conditions [1, 2, 6, 11]. This is only valid for $t >> 0$, and in order to take into account the initial times, Schnell and Turner have introduced the term of modified fractal-like kinetics [11] and they have proposed that the rate coefficient followed a temporal Zipf-Mandelbrot distribution [11]:

$$k(t) = k_0 \left(\tau + t\right)^{-h} \tag{2}$$

with the same meaning for $k_0$ and $h$ parameters as in equation (1). The new parameter, $\tau$, is a positive constant and it can be interpreted as the critical time from the rate coefficient is driven by the spatial effects [11]. Simulation data reveal a better fitting for Zipf-Mandelbrot equation [11].

As the exponential behavior is widely present for living systems, we also propose an exponential time dependence of rate coefficient:

$$k(t) = k_0[1 + h\exp\left(-\frac{t}{\tau}\right)] \tag{3}$$

This equation splits into two additive terms: the first one is $k_0$ and it is related to the rate constant in classical kinetics and the second one, $k_0 h\exp\left(-\frac{t}{\tau}\right)$ is the term due to spatial constrains. For 3D homogeneous media $h = 0$ and $k = k_0$ as in classical kinetics.

Both equations, (2) and (3), have an initial value for the rate coefficient different from $k_0$, respectively $k_0^{'}\tau^{-h}$ for Zipf-Mandelbrot equation (2) and $k_0(1+h)$ for the exponential equation (3), which can be related with the value of the diffusion-controlled rate constant that must be taken into account if the diffusion process is not anomalous [10], like in 3D homogeneous media.

## COMPUTATIONAL ALGORITHM

We perform Monte Carlo simulation for enzymatic reactions following a Michaelis-Menten mechanism and taking place in 2D unobstructed media considering only hard-sphere repulsion interactions between the molecules. The algorithm used in these simulations has been described elsewhere [8] with the difference that for this study we use 2D unobstructed lattices 100×100 with periodic boundary conditions. In order to analyze the effect of initial concentrations of substrate (S) molecules, respectively of enzyme (E) molecules we consider two major cases:

– the same initial concentration of enzyme ([E] = 0.01) and different initial concentrations of substrate: [S] = 0.1, [S] = 0.15, [S] = 0.2, [S] = 0.25 and [S] = 0.3 respectively.

– the same initial concentration of substrate molecules ([S] = 0.1) and different initial concentrations of enzyme molecules: [E] = 0.0025, [E] = 0.0050, [E] = 0.0075, [E] = 0.0100 and [E] = 0.0125 respectively.

The concentrations of reactants are expressed in number of particles divided by the lattice sites. All the particles have the same size, they occupy only one site in the lattice. For every simulation we perform 1000 time steps and 200 independent runs are computed, the results being averaged for these independent runs. The computation is implemented into in-house Fortran 77 programs.

Through these simulations we obtain the rate coefficient $k_1$ values and we perform a Levenberg-Marquardt non-linear fitting of the data using equations (2) and (3) respectively in order to obtain the values of the $h$, $\tau$, $k_0^{'}\tau^{-h}$ and $k_0(1+h)$ parameters.

## RESULTS AND DISCUSSION

For every case under consideration rate coefficient $k_1$ is time dependent and rate coefficients $k_{-1}$ and $k_{-2}$ are constant, their values corresponding to the imposed probabilities for the corresponding reactions, $g = 0.02$ and $r = 0.04$ respectively. Figure 1 shows $k_1$ rate coefficient time variations for two different initial concentrations of reactants: [E] = 0.005, [S] = 0.1 (solid line) and [E] = 0.01, [S] = 0.3 (dotted line).

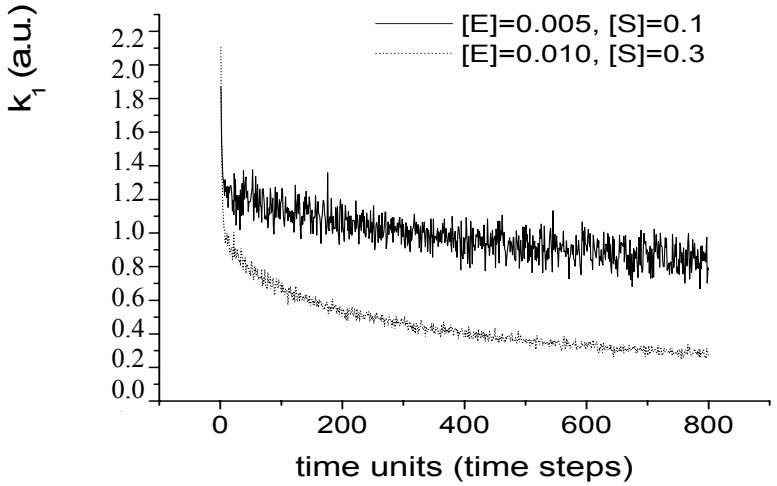


Fig. 1. Rate coefficient $k_1$ time dependence for different initial concentrations of reactants: [E] = 0.005 and [S] = 0.1 for solid line, [E] = 0.01 and [S] = 0.1 for dotted line.

In order to reveal the influence of initial concentration of reactants on reaction kinetics we analyze the values of $h$ and $\tau$ parameters in the equations (2) and (3). Figures 2.a and 2.b show $h$ parameters values for the same initial concentrations of enzyme molecules and different initial concentration of substrate (Fig. 2.a), respectively for the same initial concentration of substrate and different initial concentrations of enzyme (Fig. 2.b).

Figures 3.a and 3.b show the $\tau$ parameter values for the same initial concentrations of enzyme molecules and different initial concentrations of substrate (Fig. 3.a), respectively for the same initial concentration of substrate and different initial concentrations of enzyme (Fig. 3.b).

Zipf-Mandelbrot and the exponential equations show the increase for both $h$ and $\tau$ values with initial concentration of substrate (Figures 2.a and 3.a) and a Gaussian dependence of these parameters on initial concentration of enzyme (Figures 2.b and 3.b).

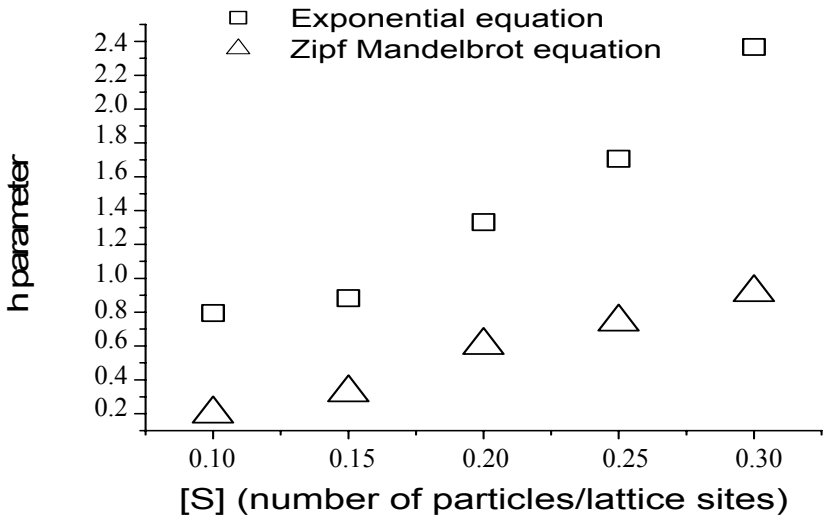


Fig. 2.a. The dependence of *h* value on initial substrate concentration ([E] = 0.01).

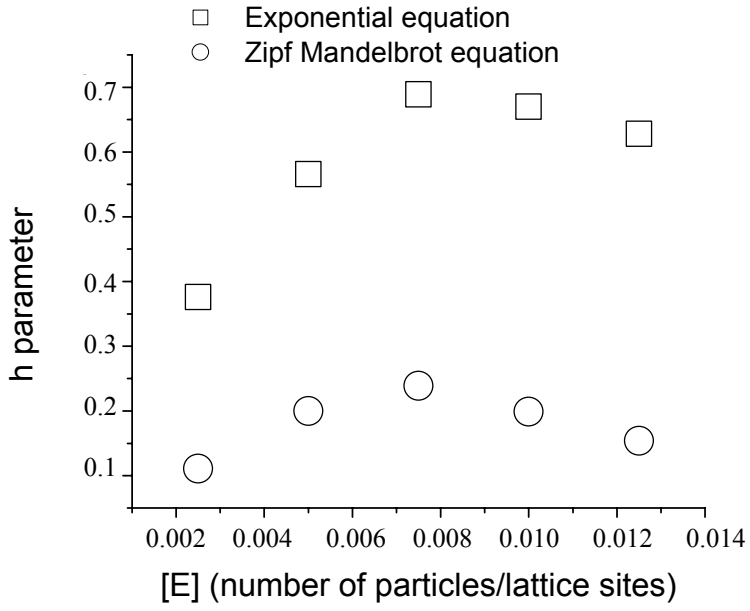


Fig. 2.b. The dependence of *h* value on initial enzyme concentration ([S] = 0.1).

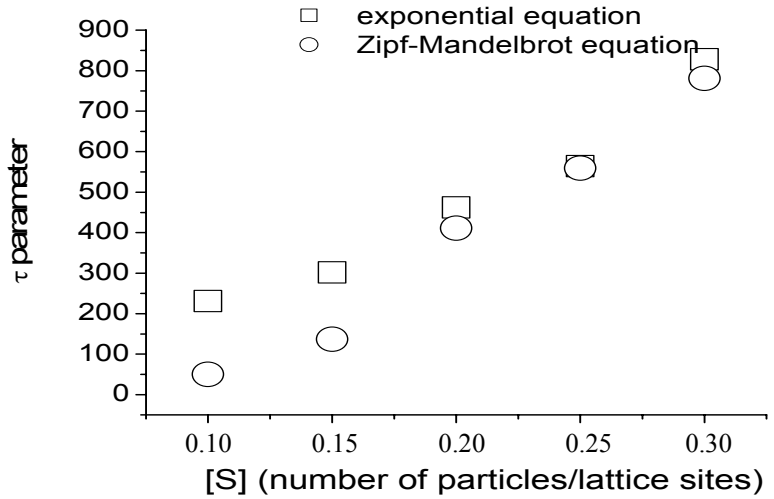


Fig. 3.a. The dependence of τ value on initial substrate concentration ([E] = 0.01).

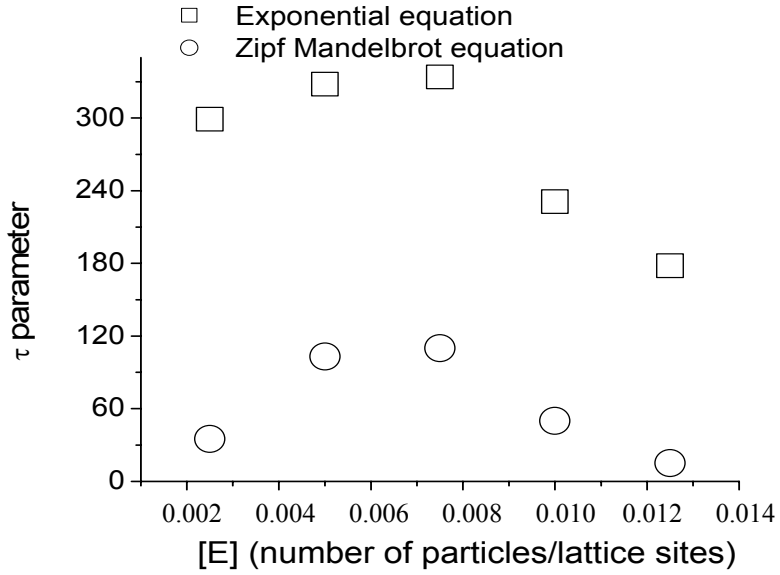


Fig. 3.b. The dependence of τ value on initial enzyme concentration ([S] = 0.01).

*Table 1*

The dependence of initial values of rate coefficients in equations (2) and (3) on initial concentrations of reactants

| Case | Concentration (number of particles/ lattice sites) | $k_0'\tau^{-h}$ (Eq. 2, a.u.) | $k_0(1+h)$ ( Eq. 3, a.u) |
|---|---|---|---|
| [E] = 0.01, different [S] | [S]=0.1 | 1.364 ± 0.014 | 1.289 ± 0.007 |
| | [S]=0.15 | 1.387 ± 0.012 | 1.349 ± 0.019 |
| | [S]=0.2 | 1.402 ± 0.012 | 1.382 ± 0.064 |
| | [S]=0.25 | 1.452 ± 0.013 | 1.426 ± 0.088 |
| | [S]=0.3 | 1.475 ± 0.018 | 1.436 ± 0.056 |
| [S] = 0.1, different [E] | [E]=0.0025 | 1.116 ± 0.007 | 1.449 ± 0.015 |
| | [E]=0.005 | 1.124± 0.017 | 1.268 ± 0.017 |
| | [E]=0.0075 | 1.126 ± 0.011 | 1.278 ± 0.017 |
| | [E]=0.01 | 1.132 ± 0.056 | 1.247 ± 0.011 |
| | [E]=0.0125 | 1.127 ± 0.018 | 1.305 ± 0.017 |

The value of diffusion-controlled rate constant in 3D homogeneous media does not depend on initial concentrations of reactants. As explained in the subchapter *Diffusion controlled reactions in two-dimensional media,* in 2D homogeneous media, the diffusion is anomalous, and the initial value of the rate coefficient must have a similar meaning with a true rate constant, $k_0$. In order to see this, we analyze the dependence of the parameters $k_0'\tau^{-h}$ in Zipf-Mandelbrot equation respectively of $k_0(1+h)$ in the exponential equation with initial concentrations of reactants. These data are presented in Table 1, and we can deduce that these values are more or less constant with similar values, being 1.35±0.10 the mean value from the exponential equation, which are independent of the initial concentrations either of substrate or of the enzyme. For the Zipf-Mandelbrot equation, this is still not true, because the mean value of this initial rate constant is different, 1.42±0.06, from averaging the values obtained for different [S], and 1.22±0.10 for different [E].

## CONCLUSIONS

The results presented here reveal non-classical kinetics for Michaelis-Menten enzymatic reactions taking place in two dimensional media. As we perform simulations in 2D unobstructed lattices, this phenomenon is strongly related to the reduced spatial dimensionality of the media resulting in a small degree of mixing of reactants. Our results are in good qualitative agreement with other simulation data revealing fractal kinetics of chemical reactions in 2D obstructed and unobstructed media [2, 6, 11].

This non-classical kinetics could be described using Zipf-Mandelbrot equation, respectively using an exponential function, both these equations giving almost the same degree of correlation between simulation data and fitted values and showing the same qualitative behavior for the parameters they contain. In our opinion, the exponential function is much more advantageous to describe fractal kinetics for this case. We sustain this affirmation also by the fact that the initial value obtained for $t \to 0$ in the exponential equation, $k_0(1+h)$, is independent of the initial concentrations either of substrate or of the enzyme, which is not true for the initial value, $k_0^{'}\tau^{-h}$, in the Zipf-Mandelbrot equation.

Within this paper we also analyze the effects of initial concentrations of reactants on reaction rates for enzymatic reactions taking place in unobstructed 2D media. For the same initial concentration of enzyme, the increase of concentration of substrate results in increasing fractality. It is reflected by a linear increase of the values of $h$ and $\tau$ parameters with increasing substrate concentration. The reason of this behavior is that as substrate concentration is higher, the lattice is more crowded and the diffusion of reactants is hindered. Hindered diffusion of reactants also decreases rate coefficients of diffusion controlled reactions. For the same initial concentration of substrate, increasing initial concentration of enzyme seems to have two opposite effects: for small [E] values it negatively affects rate coefficients but for high [E] values, the effect is positive. In case of small [E] values, substrate molecule must explore large regions of space before reacting and it decreases their reactivity, reflected by increasing $h$ and $\tau$ parameters values. For large [E] values the substrate reaches more easily the enzyme increasing the reactivity, reflected by decreasing $h$ and $\tau$ parameter values.

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